

# Probing the Conformations of Single Molecule via Photon Counting Statistics

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We suggest an approach to detect the conformation of single molecule by using the photon counting statistics. The generalized Smoluchowski equation is employed to describe the dynamical process of conformational change of single molecule. The resonant trajectories of the emission photon numbers  $\langle N \rangle$  and the Mandel's  $Q$  parameter, in the space of conformational coordinates  $\mathcal{X}$  and frequency  $\omega_L$  of external field ( $\mathcal{X} - \omega_L$  space), can be used to rebuild the conformation of the single molecule. As an example, we consider Thioflavin T molecule. It demonstrates that the results of conformations extracted by employing the photon counting statistics is excellent agreement with the results of *ab initio* computation.

## I. INTRODUCTION

The single molecule technique, excluding the ensemble average compared with traditional techniques, can be used to detect and measure the dynamical process in the level of single molecule [1]. This technique could help us to discover some new quantum phenomena occurred in single molecule level, such as, spectral diffusion, fluorescence intermittency [1, 2] *etc.*. With the advancement of the experimental techniques, the single molecule technique has become one of the useful techniques for studying physical, chemical and material science. However, there are some difficulties in the single molecule experiments. One of the major difficulties is the conformational dynamics strongly effects the single molecule signals under different conditions.

The conformational information of single molecule plays an important role in physical, chemical and biological processes. For example, the conformational changes of some organic molecules can affect the efficient of the solar cells [3–5]. Also, neurodegenerative Alzheimer's and Parkinson's diseases, cataracts associate with unnormal conformation of the macromolecules [6–11]. Several methods are developed to study the conformational changes of single molecules experimentally and theoretically, such as, single pair fluorescence resonance energy transfer (sp-FRET) [12], single molecule fluorescence polarization anisotropy (sm-FPA), and atomic force microscopy [13], molecular dynamics simulation [14, 15], Monte Carlo simulation [16], and kinetic method [17–22] *etc.*. In these methods, it is assumed that the single molecule transits between some discrete states for investigating the single molecule conformational dynamics. In the simulation and the kinetic methods, the transition rate constants between different states are obtained via the best fitting experimental data. However, in some cases, the conformational changes of single molecule show complex behaviors since the conformational changes of single molecule depend on its

conformational coordinates  $\mathcal{X}$ . The methods mentioned above are hardly to completely describe the conformational changes with the case that the single molecule is changing with the conformational coordinates  $\mathcal{X}$ .

To overcome this inadequacy, one develops different methods to simulate the conformations numerically, and compares the numerical simulation conformation with in-direct experimental results. The question, however, is that can we direct probe the conformations of single molecule experimentally, and how can we do this? As we know, the emission photons from the single molecule not only include the information of the molecule interaction with its surrounding environments but also include the information of the single molecule itself. Such as, the spectral diffusion process of the single molecule reflects the dynamics of the single molecule interaction with its surrounding molecules [1, 2, 23, 24], and the blinking phenomenon in the single quantum dots reflects the details of quantum process which occurred in the systems. Experimentally, the factorial moments of emitted photons  $\langle N_r \rangle$ , arrival times and frequencies of emitted photons *etc.* are serving as the experimental data, which contain information about the nature of conformations and time scale of the underlying conformational changes. In this paper, we suggest an approach to extract the conformational information of single molecule in the space of the conformational coordinate and the frequency of external field ( $\mathcal{X} - \omega_L$  space) from the resonant trajectory of emitted photon. As a concrete example, we take the single molecule of Thioflavin T to demonstrate the application of our theoretical approach extracting its conformation numerically.

## II. THEORETICAL FRAMEWORK

We assume a single molecule has several energy levels  $\{|n(\mathcal{X})\rangle\}$ , and its corresponding eigen-energy is  $\epsilon_n(\mathcal{X})$ , where  $\mathcal{X}$  is the conformational coordinate. In the energy picture of the single molecule,  $\epsilon_n(\mathcal{X})$  can also be thought as the “energy surface”. The conformational dynamics of the single molecule can be thought as the

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diffusion in different energy surfaces. It hence could be assumed the single molecular system satisfies the generalized Smoluchowski equation

$$\frac{\partial}{\partial t}\rho(\mathcal{X},t) = -\frac{i}{\hbar}[\mathcal{H},\rho(\mathcal{X},t)] + \mathcal{Z}\rho(\mathcal{X},t) + \mathcal{R}\rho(\mathcal{X},t) + \mathcal{L}\rho(\mathcal{X},t), \quad (1)$$

where  $\mathcal{H}$  is the Hamiltonian of the single molecular system, including the Hamiltonian of “bare” single molecule and the interaction between the single molecule and external field,  $\mathcal{Z}\rho$  describes the conformational “diffusion” process of the single molecule,  $\mathcal{R}\rho$  describes the transitions caused by the system interacting with it surrounding environments, and  $\mathcal{L}\rho$  describes the spontaneous emission process.  $\mathcal{R}$  and  $\mathcal{L}$  are the environment assistant transition and spontaneous emission operators, respectively [25].

The operator  $\mathcal{Z}$  is the Smoluchowski operator, it can, via its acting on density matrix, be defined as

$$(\mathcal{Z}\rho)_{nm} = \frac{1}{2}\frac{\partial}{\partial\mathcal{X}}De^{-\epsilon_n(\mathcal{X})}\frac{\partial}{\partial\mathcal{X}}e^{+\epsilon_n(\mathcal{X})}\rho_{nm} + \frac{1}{2}\frac{\partial}{\partial\mathcal{X}}De^{-\epsilon_m(\mathcal{X})}\frac{\partial}{\partial\mathcal{X}}e^{+\epsilon_m(\mathcal{X})}\rho_{nm}, \quad (2)$$

where  $D$  is the diffusion coefficient.

The Langevin equation can be employed to determine the surface  $\epsilon_1(\mathcal{X})$  of single molecule in ground state. The surface of single molecule in ground state can be expressed as

$$\epsilon_1(\mathcal{X}) = \epsilon_1(\mathcal{X}_e) + \frac{1}{D}\int_{\mathcal{X}}^{\mathcal{X}_e}\dot{r}_1dr_1, \quad (3)$$

where  $\mathcal{X}_e$  is the equilibrium position. For convenient, we can assume  $\epsilon_1(\mathcal{X}_e) = 0$ .

The single molecule can, using the precise tuning capability, be pumped by scanning microscopy. It is experimentally imaging the resonant excitation and photon emission. That is, we can obtain resonant peaks from the emitted photon counting statistics of first factorial moment  $\langle N \rangle$  or the second factorial moment of the Mandel's  $Q$  parameter for long time limit. Each of the resonant peaks of photon counting statistics corresponds to an resonant absorption between the different conformational excited states and ground state of single molecule in  $\mathcal{X} - \omega_L$  space.

If we denote  $\hbar\omega_{mn}^{(r)}(\mathcal{X})$  as the resonant absorption peak between the states  $|m\rangle$  and  $|n\rangle$  in  $\mathcal{X} - \omega_L$  space. Or, we define  $\omega_{mn}^{(r)}(\mathcal{X})$  as a resonant trajectory of emitted photon for long time limit in  $\mathcal{X} - \omega_L$  space, we have

$$\hbar\omega_{mn}^{(r)}(\mathcal{X}) = \epsilon_m(\mathcal{X}) - \epsilon_n(\mathcal{X}). \quad (4)$$

Then, one can obtain the conformation of  $\epsilon_m(\mathcal{X})$  from the resonant trajectory  $\hbar\omega_{mn}^{(r)}(\mathcal{X})$  and the known conformation of  $\epsilon_n(\mathcal{X})$ . The conformation of  $\epsilon_m(\mathcal{X})$  can be expressed as following

$$\epsilon_m(\mathcal{X}) = \hbar\omega_{mn}^{(r)}(\mathcal{X}) + \epsilon_n(\mathcal{X}), \quad (5)$$

by employing a resonant trajectory.

### III. AN EXAMPLE

We numerically demonstrate that the resonant trajectories of photon counting moments are employed to extract the conformational changes of the single Thioflavin T (ThT) molecule. The fluorescence intensity change of the ThT molecule is considered associating with its conformational changes, which can be used to detect the dynamics of the amyloid fibrils [26]. In this paper, we consider the case that the conformation of ThT molecule is changed with a torsion angle  $\varphi$ , which is the angle between the benzothiazole and the dimethylaminobenzene rings (for ThT molecule, the conformational changes can also be thought as configurational changes). Namely, in this case we have  $\mathcal{X} = \varphi$ . The ThT molecule can be described by four levels [27–29]: two conformational ground states  $|1\rangle$  and  $|2\rangle$ , and two conformational excited states  $|3\rangle$  and  $|4\rangle$ . The transitions between the ground state  $|1\rangle$  ( $|2\rangle$ ) to the excited states  $|3\rangle$  and  $|4\rangle$  are dipole transition allowed. The transition between conformational ground (excited) states  $|2\rangle$  and  $|1\rangle$  ( $|4\rangle$  and  $|3\rangle$ ) is dipole transition forbidden.

The Hamiltonian of the single molecule can be expressed as

$$\mathcal{H} = \mathcal{H}_0 + \mathcal{H}', \quad (6)$$

where  $\mathcal{H}_0$ , the Hamiltonian of the “bare” single molecule, can be expressed as

$$\mathcal{H}_0 = \sum_{n=1}^4 \epsilon_n(\varphi)|n\rangle\langle n|, \quad (7)$$

where  $\epsilon_n(\varphi) = \hbar\omega_n(\varphi)$  is the eigen-energy. The interaction between the single molecule and the external field reads

$$\mathcal{H}' = -\sum_{mn} \mu_{mn} \cdot \mathcal{E}_0(t) \cos(\omega_L t) (a_{mn}^\dagger + a_{mn}), \quad (8)$$

where  $a_{mn}^\dagger = |m\rangle\langle n|$ , and  $a_{mn} = |n\rangle\langle m|$ , with  $m = 3, 4$  and  $n = 1, 2$ ,  $\mu_{mn}$  is the transition dipole between the states  $|m\rangle$  and  $|n\rangle$ ,  $\mathcal{E}_0(t)$  and  $\omega_L$  are the amplitude and the angular frequency of the external field, respectively.

Based on Eqs. (7) and (8), the Hamiltonian (6) of the single molecule in the representation of the “bare” single molecule  $\mathcal{H}_0$  and under the rotating wave approximation (RWA), can be written as

$$\mathcal{H}(\varphi) = \hbar \begin{pmatrix} 0 & 0 & \Omega_{31}(t)/2 & \Omega_{41}(t)/2 \\ 0 & \omega_{21} & \Omega_{32}(t)/2 & \Omega_{42}(t)/2 \\ \Omega_{31}(t)/2 & \Omega_{32}(t)/2 & -(\Delta + \omega_{43}) & 0 \\ \Omega_{41}(t)/2 & \Omega_{42}(t)/2 & 0 & -\Delta \end{pmatrix}, \quad (9)$$

where  $\Delta = \omega_L - \omega_{41}$  is the detuning frequency between external field  $\omega_L$  and the transition frequency

$\omega_{41} = \omega_4(\varphi) - \omega_1(\varphi)$ ,  $\Omega_{nm}(t) = -\mu_{nm} \cdot \mathcal{E}_0(t)/\hbar$  is the Rabi frequencies.

To obtain the resonant absorption trajectory of photon counting moments of  $\langle N \rangle(\varphi, \omega_L)$  and the Mandel's  $Q(\varphi, \omega_L)$  parameter in the  $\varphi - \omega_L$  space, the generating function approach is employed to simulate the trajectories of  $\langle N \rangle(\varphi, \omega_L)$  and  $Q(\varphi, \omega_L)$  for long time limit theoretically. As noted in previous works [2, 20, 23–25, 30–33], the message taken with the emitted photon for long time limit from single molecules is the information when the single molecule is being excited. Hence, we could note down the scenario of the conformation of single molecule on the time of being excited using the resonant trajectory in  $\mathcal{X} - \omega_L$  space for long time limit via the generating function approach of photon counting statistics. We can scan the single molecule to obtain the resonant trajectory in  $\mathcal{X} - \omega_L$  space by employing the (ultra)fast laser (we mean here the laser is faster than the conformational dynamics of single molecule) to pump the single molecule.

The generating function is defined as follows [2, 23, 24, 32, 33],

$$\mathcal{G}(\varphi, s, t) = \sum_n \rho^{(n)}(\varphi, t) s^n, \quad (10)$$

where  $\sum_{n=0}^{\infty} \rho^{(n)}(\varphi, t) = \rho(\varphi, t)$ .  $\rho^{(n)}(\varphi, t)$  is the partition of single molecule system emitted  $n$  photons at the time interval  $[0, t]$  in the conformation  $\varphi$ ,  $s$  is the auxiliary parameter for counting photons. The generating function, based on the generalized Smoluchowski equation (1) and after some algebra using Eq. (10), satisfies the following equation (The exciting process is great faster than the conformational dynamics by using ultra-fast laser field. Here, we omit the conformational operator in our equation.)

$$\begin{aligned} \frac{\partial}{\partial t} \mathcal{G}(\varphi, s, t) = & -\frac{i}{\hbar} [\mathcal{H}(\varphi, t), \mathcal{G}(\varphi, s, t)] + \sum_{m,n} (\mathcal{Z}\mathcal{G}(\varphi, s, t))_{mn} \\ & + \sum_{m,n} \frac{\Gamma_{mn}}{2} \left( 2sa_{mn}\mathcal{G}(\varphi, s, t)a_{mn}^\dagger - \right. \\ & \left. a_{mn}^\dagger a_{mn}\mathcal{G}(\varphi, s, t) - \mathcal{G}(\varphi, s, t)a_{mn}^\dagger a_{mn} \right) \\ & + \sum_{m,n} \frac{\mathcal{R}_{mn}}{2} \left( 2a_{mn}\mathcal{G}(\varphi, s, t)a_{mn}^\dagger - \right. \\ & \left. a_{mn}^\dagger a_{mn}\mathcal{G}(\varphi, s, t) - \mathcal{G}(\varphi, s, t)a_{mn}^\dagger a_{mn} \right), \quad (11) \end{aligned}$$

where  $\Gamma_{mn}$  is the spontaneous emission rate between the states  $|m\rangle$  and  $|n\rangle$ ,  $\mathcal{R}_{mn}$  is the environment assistant transition rate between the states  $|m\rangle$  and  $|n\rangle$ .

The factorial moments of  $\langle N_r \rangle$  can be simply obtained by taking derivatives with respect to  $s$  evaluated at  $s = 1$ , namely

$$\begin{aligned} \langle N_r \rangle(\varphi, t) & \equiv \langle N(N-1)(N-2) \cdots (N-r+1) \rangle (12) \\ & = \frac{\partial^r}{\partial s^r} \mathcal{Y}(\varphi, s, t) \Big|_{s=1}, \end{aligned}$$

and the Mandel's  $Q(\varphi, t)$  parameter calculated as

$$Q(\varphi, t) = \frac{\langle N_2 \rangle(\varphi, t) - \langle N_1 \rangle^2(\varphi, t)}{\langle N_1 \rangle(\varphi, t)}, \quad (13)$$

where the working generating function  $\mathcal{Y}(\varphi, s, t)$  is defined as follows

$$\mathcal{Y}(\varphi, s, t) = \sum_{n=1}^4 \mathcal{G}_{nn}(\varphi, s, t). \quad (14)$$

Scanning the single molecule system, we can obtain the resonant trajectories of the mean number of photons  $\langle N \rangle(\varphi, \omega_L) = \langle N_1 \rangle(\varphi, \omega_L)$  and  $Q(\varphi, \omega_L)$  for long time  $t$  in  $\varphi - \omega_L$  space.

In our numerical simulations, the spontaneous emission rate  $\Gamma_{mn}$  is existence for  $m = 3, 4$  and  $n = 1, 2$ ; and only the environment assistant transition rates  $\mathcal{R}_{21}$  and  $\mathcal{R}_{43}$  are existence. For ThT molecule, the conformational dynamics (the corresponding time scale  $\tau_c \sim 10^{-11} - 10^{-9}$  s) is great faster than the spontaneous emission transition (the corresponding time scale  $\tau_f \sim 10^{-8}$  s). One can employ an ultra-short laser pulse to excite the single molecule, and the width of the laser pulse  $T$  would less shorter than conformational dynamical time scale  $\tau_c$ . As we know, a finite length laser wave could cause the laser frequency has a boarden  $\delta\omega \sim 1/T$ . If  $\delta\omega$  is larger or the same as the energy gap between two excited states or ground states, the states can not be distinguished. The width of laser pulse  $T$  can be chosen as  $10^{-14} \leq T \leq 10^{-11}$ , and the pulse area  $\Theta_{mn} = \int_{-\infty}^{\infty} \Omega_{mn}(t) dt \gg \pi$ , which could promise the exciting process is great faster than conformational dynamics. In our calculation, we choose the pulse area  $\Theta_{mn} = 100\pi$ .

Figure 1 numerically simulates the possible experimental results of the resonant trajectories of emission photons  $\langle N \rangle$  and the Mandel's  $Q$  parameter in the  $\varphi - \omega_L$  (conformational-frequency) space. The spontaneous emission rates  $\Gamma_{41} = \Gamma_{42} = \Gamma_{31} = \Gamma_{32} = \gamma = 3 \times 10^8$  Hz, the Rabi frequency  $\Omega_{14} = \Omega_{13} = \Omega_{24} = \Omega_{23} = 10^4 \gamma$  and the environment assistant transition rates  $\mathcal{R}_{21} = \mathcal{R}_{43} = \gamma$  [34], which corresponds to relaxation between the excited states  $\epsilon_4(\varphi)$  and  $\epsilon_3(\varphi)$  and the ground states  $\epsilon_2(\varphi)$  and  $\epsilon_1(\varphi)$ . The resonant trajectories of the emission photons  $\langle N \rangle(\varphi, \omega_L)$  and the Mandel's  $Q(\varphi, \omega_L)$  in Fig. 1 correspond to the resonant absorption transition between the single molecule energy levels. In the Mandel's  $Q(\varphi, \omega_L)$  panel of Fig. 1, trajectory 1 corresponds to the resonance transition  $|4\rangle \rightarrow |1\rangle$ ; trajectory 2 corresponds to the transition  $|3\rangle \rightarrow |1\rangle$ ; trajectory 3 corresponds to the transition  $|4\rangle \rightarrow |2\rangle$ ; trajectory 4 corresponds to the transition  $|4\rangle \rightarrow |2\rangle$ . That means (we denote the curves frequency by  $\omega_{tn}(\varphi)$ , ( $n = 1, 2, 3, 4$ ))  $\omega_{11}(\varphi) = \omega_4(\varphi) - \omega_1(\varphi)$ ,  $\omega_{12}(\varphi) = \omega_3(\varphi) - \omega_1(\varphi)$ ,  $\omega_{13}(\varphi) = \omega_4(\varphi) - \omega_2(\varphi)$ , and  $\omega_{14}(\varphi) = \omega_3(\varphi) - \omega_2(\varphi)$ . Then, we can extract the conformational information of the single molecule:  $\omega_4(\varphi) = \omega_{11}(\varphi) + \omega_1(\varphi)$ ,  $\omega_3(\varphi) = \omega_{12}(\varphi) + \omega_1(\varphi)$ , and  $\omega_2(\varphi) = \omega_{13}(\varphi) + \omega_1(\varphi)$ .

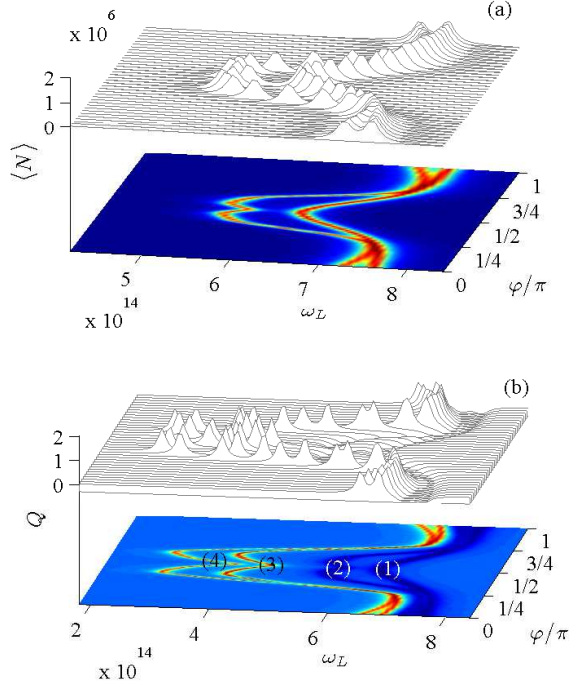


FIG. 1. The resonant trajectories of emission photon numbers  $\langle N \rangle$  and the Mandel's  $Q$  parameter in the conformational coordinate and frequency space (torsion angle  $\varphi$  and the laser frequency  $\omega_L$ ). The Rabi frequency  $\Omega_{14} = \Omega_{24} = \Omega_{13} = \Omega_{23} = 10^4\gamma$ , the spontaneous emission rates  $\Gamma_{41} = \Gamma_{42} = \Gamma_{31} = \Gamma_{32} = \gamma$ , and environment assistant transition rates  $\mathcal{R}_{21} = \mathcal{R}_{43} = \gamma$ .

Figure 2 demonstrates the numerical results of the conformations of the ThT single molecule which is extracted using the resonant trajectories (shown in Fig. 1) of the emission photons  $\langle N \rangle(\varphi, \omega_L)$  (a) and Mandel's  $Q(\varphi, \omega_L)$  parameter (b).

It should be noted that, as shown in the top panel of Fig. 2, we can not obtain the surface  $\epsilon_2(\varphi)$  of single molecule from the resonant trajectory of the mean number of photons  $\langle N \rangle(\varphi, \omega_L)$ . The reason is that the surface of state  $|2\rangle$  is more unstable than state  $|1\rangle$  for the ThT molecule, the population of state  $|2\rangle$  is less than that of state  $|1\rangle$ , and the probability of the system transition from state  $|2\rangle$  into states  $|3\rangle$  and  $|4\rangle$  is very small. This results in the resonant trajectories  $\langle N \rangle(\varphi, \omega_L)$  of the transition from state  $|2\rangle$  into states  $|3\rangle$  and  $|4\rangle$  are very weak (see top panel of Fig. 1). However, the resonant trajectory of the Mandel's  $Q$  parameter, related to the second factorial moment, as another probing signal is strong enough. As shown in the bottom panel of Fig. 1, the resonant trajectory of the Mandel's  $Q$  parameter represents all the conformational structures. As the comparison, we also show the results of conformation of ThT by employing *ab initio* simulations of Ref. [27], the results of *ab initio* simulations are marked using

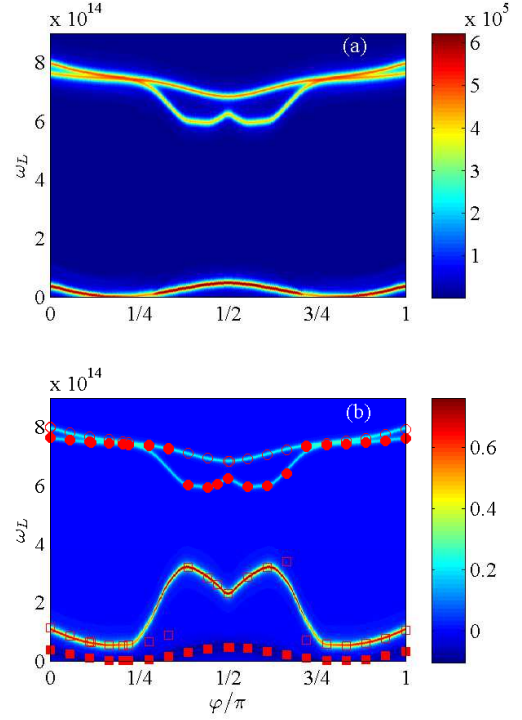


FIG. 2. (Color Online) The conformational structures of the ThT single molecule. The conformation of the single Th T molecule is increased by the resonant trajectories in the conformational coordinate and frequency space shown in Fig. 1. The marks are the results of *ab initio*. [27]

circles, solid-circles, squares and solid-squares, respectively. The results are excellent agreement with each other.

#### IV. CONCLUDING REMARK

In this paper, we demonstrate an approach obtaining single molecule conformational structures via photon counting statistics. The Langevin and generalized Smoluchowski equations are employed to describe the conformational dynamics. The resonant trajectories of the emission photon numbers  $\langle N \rangle$  and Mandel's  $Q$  parameter in conformational-frequency space ( $\mathcal{X} - \omega_L$ ) include all the conformational information of single molecule. The single molecule conformational structures can be obtained from the resonant trajectory in ( $\mathcal{X} - \omega_L$ ) space. The ThT molecule as an example is demonstrated, and the results are excellent agreement with that of *ab initio*.



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- [1] E. Barkai, Y. Jung, and R. Silbey, *Annu. Rev. Phys. Chem.* **55**, 457 (2004).
- [2] Y. Zheng and F. L. H. Brown, *Phys. Rev. Lett.* **90**, 238305 (2003).
- [3] Y. Li, K. Gao, Z. Sun, S. Yin, D. Liu, and S. Xie, *Phys. Rev. B* **78**, 014304 (2008).
- [4] K. Gao, S. Xie, S. Yin, and D. Liu, *Organic Electronics* **13**, 784 (2012).
- [5] K. Gao, X. Liu, D. Liu, and S. Xie, *Phys. Rev. B* **75**, 205412 (2007).
- [6] D. J. Selkoe, *Nature* **426**, 900 (2003).
- [7] E. Zerovnik, *Eur. J. Biochem* **269**, 3362 (2002).
- [8] J. D. Harper, C. M. Lieber, and P. T. Lansbury, Jr., *Chem. Biol.* **4**, 951 (1997).
- [9] R. W. Carrell and B. Cooptu, *Curr. Opin. Struct. Biol.* **8**, 799 (1998).
- [10] E. H. Koo, P. T. Lansbury, Jr., and J. W. Kelly, *Proc. Natl. Acad. Sci., U.S.A* **96**, 9989 (1999).
- [11] J. L. Guo, D. J. Covell, J. P. Daniels, M. Iba, A. Stieber, B. Zhang, D. M. Riddle, L. K. Kwong, Y. Xu, J. Q. Trojanowski, and V. M. Y. Lee, *Cell* **154**, 103 (2013).
- [12] S. Weiss, *Nat Struct Biol* **7**, 724 (2000).
- [13] A. Agnihotri and C. A. Siedlecki, *Langmuir* **20**, 8846 (2004).
- [14] V. A. Voelz, G. R. Bowman, K. Beauchamp, and V. S. Pande, *J. Am. Chem. Soc.* **132**, 1526 (2010).
- [15] Y. Liu, J. Strümpfer, P. L. Freddolino, M. Gruebele, and K. Schulten, *J. Phys. Chem. Lett.* **3**, 1117 (2012).
- [16] H. Senderowitz, F. Guarnierl, and W. C. Still, *J. Am. Chem. Soc.* **117**, 8211 (1995).
- [17] S. Presse, J. Lee, and K. A. Dill, *J. Phys. Chem. B* **117**, 495 (2013).
- [18] H. Neuweiler, C. M. Johnson, and A. R. Rersht, *Proc. Natl. Acad. Sci., U.S.A.* **106**, 18569 (2010).
- [19] N. Agmon, *J. Phys. Chem. B* **104**, 7830 (2000).
- [20] Y. Zheng and F. L. H. Brown, *J. Chem. Phys.* **121**, 3238 (2004).
- [21] J. Cao, *J. Phys. Chem. B* **110**, 19040 (2006).
- [22] J. Cao and R. J. Silbey, *J. Phys. Chem. B* **112**, 12867 (2008).
- [23] Y. Zheng and F. L. H. Brown, *J. Chem. Phys.* **119**, 11814 (2003).
- [24] Y. Zheng and F. L. H. Brown, *J. Chem. Phys.* **139**, 164120 (2013).
- [25] G. Bel, Y. Zheng, and F. L. H. Brown, *J. Phys. Chem. B* **110**, 19066 (2006).
- [26] H. I. LeVine, *Protein Sci.* **2**, 404 (1993).
- [27] V. I. Stsiapura, A. A. Maskevich, V. A. Kuzmitsky, K. K. Turoverov, and I. M. Kuznetsova, *J. Phys. Chem. A* **111**, 4829 (2007).
- [28] V. I. Stsiapura, A. A. Maskevich, V. A. Kuzmitsky, V. N. Uversky, I. M. Kuznetsova, and K. K. Turoverov, *J. Phys. Chem. B* **112**, 15893 (2008).
- [29] V. I. Stsiapura, A. A. Maskevich, S. A. Tikhomirov, and O. V. Bugarov, *J. Phys. Chem. A* **114**, 8345 (2010).
- [30] E. Barkai, Y. Jung, and R. Silbery, *Phys. Rev. Lett.* **87**, 207403 (2001).
- [31] Y. He and E. Barkai, *Phys. Rev. A* **74**, 011803(R) (2006).
- [32] Y. He and E. Barkai, *Phys. Rev. Lett.* **93**, 068302 (2004).
- [33] S. Mukamel, *Phys. Rev. A* **68**, 063821 (2003).
- [34] The transition rates  $\mathcal{R}_{21}$  and  $\mathcal{R}_{43}$  can be considered the single molecule colliding with solution molecules. The collision life time  $\tau_0$  can be expressed as  $\frac{1}{\tau_0} = 4d^2n\sqrt{\frac{\pi k_B T}{M}}$  [35], where  $d$  is the distant between the molecules,  $n$  is the molecule number density,  $k_B$  is Boltzmann's constant,  $T$  is the temperature, and  $M$  is the molecular mass. For the single molecule experiments at room temperature, the density  $n \in (1, 10^6)$  n mol/L [36]. The corresponding life time  $\tau_0 \in (10^{-3}, 10^{-9})$  s. In our calculation, we choose  $1/\tau_0 = 3 \times 10^8$  Hz.
- [35] R. Loudon, *The Quantum Theory of Light*, 3rd ed. (Oxford University Press, New York, 2000).
- [36] P. R. Selvin and T. Ha, *Single Molecule Techniques: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, New York, 2008).